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Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

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Citation (APA):
Ley, D., Kazemi Seresht, A., Engmark, M., Magdenoska, O., Nielsen, K. F., Kildegaard, H. F., & Andersen, M. R. (2016). *Multi-omic profiling of EPO producing Chinese hamster ovary cell panel reveals metabolic adaptation to heterologous protein production*. Poster session presented at Cell Culture Engineering XV, La Quinta, CA, United States.

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Multi-omic profiling of EPO producing Chinese hamster ovary cell panel reveals metabolic adaptation to heterologous protein production

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Introduction

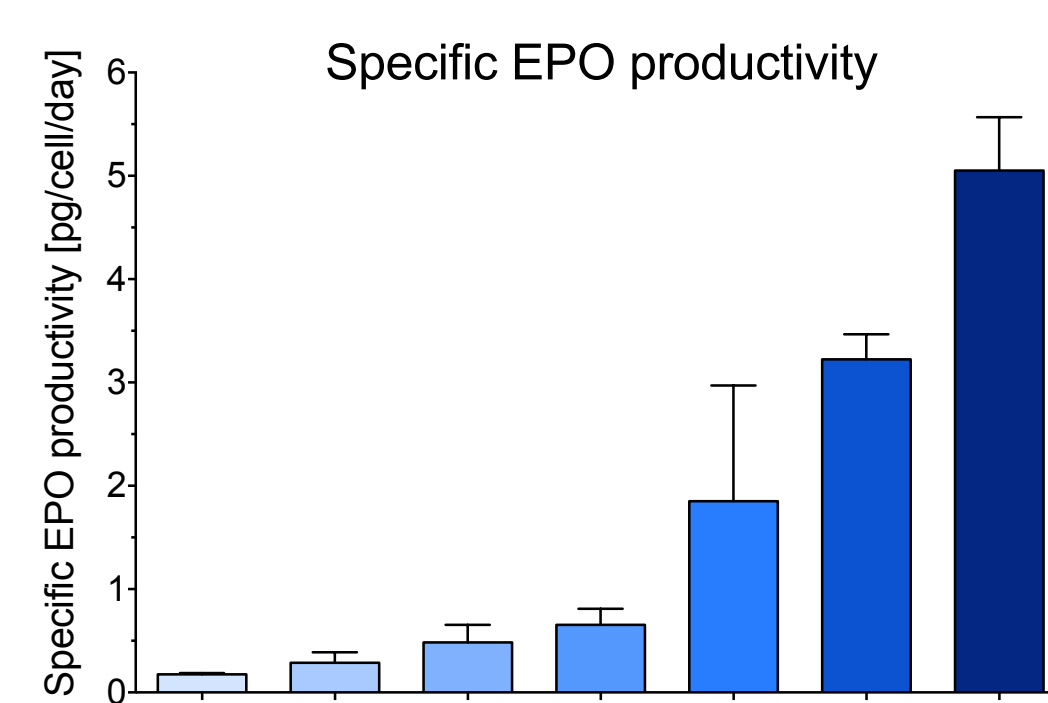
Heterologous protein production in CHO cells imposes a burden on the host cell metabolism and impact cellular physiology on a global scale. In this work, a multi-omics approach was applied to characterize the physiological impact of erythropoietin production, and discover production bottlenecks, in a panel of CHO-K1 cells in batch and chemostat culture.

Objectives

- Generate a panel of EPO producing clones with broad specific protein productivity.
- Identify the production bottleneck in the secretory protein production pathway.
- Characterize the burden of EPO production on the host cell metabolism and identify bottlenecks in energy metabolism.

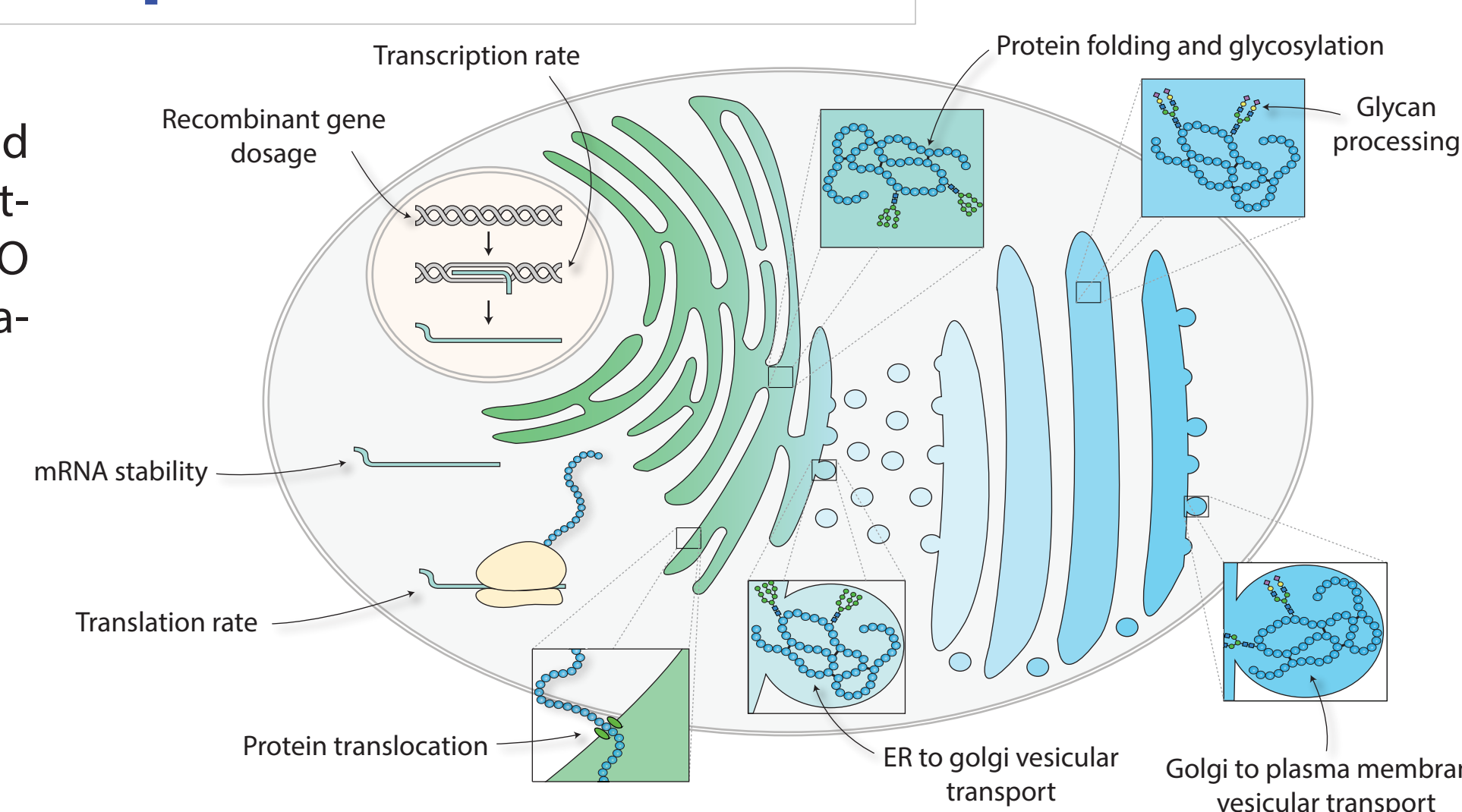
Cell lines

The panel consist of 7 EPO-producing CHO-K1 clones spanning a 25-fold productivity range.



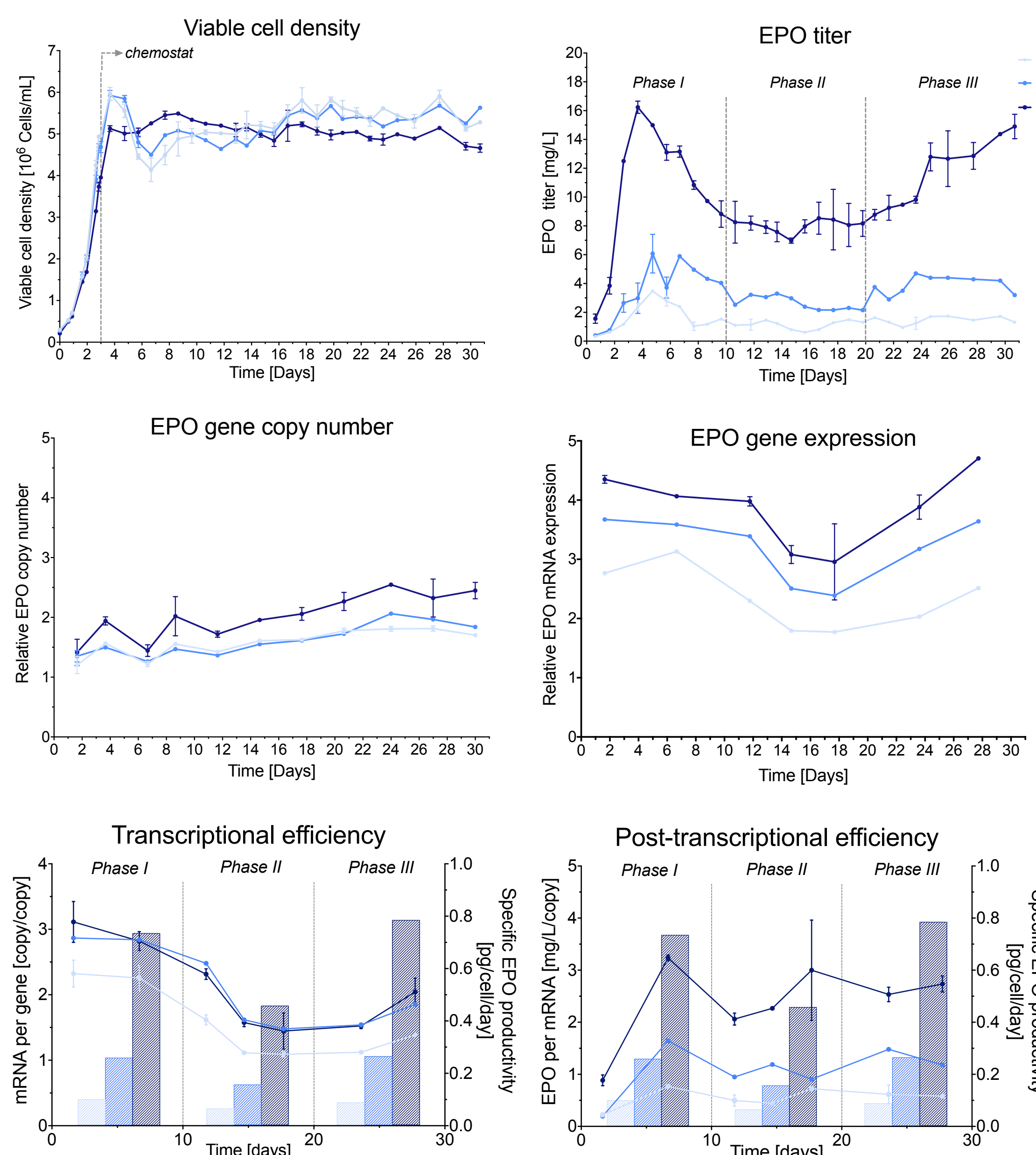
Common protein expression bottlenecks

Common bottlenecks found in production of secreted heterologous proteins in CHO cells, as reported in the literature^{1,2,3,4,5,6,7}.



Investigating the protein expression bottleneck

To identify the protein expression bottleneck, we cultured three clones (C1, C4 and C7) in chemostat culture for 31 days and determined EPO gene copy numbers, mRNA levels and secreted EPO. The post-transcriptional efficiency (ratio of EPO per mRNA) indicated a production bottleneck enrooted downstream of transcription in clone 1 and 4 relative to clone 7.



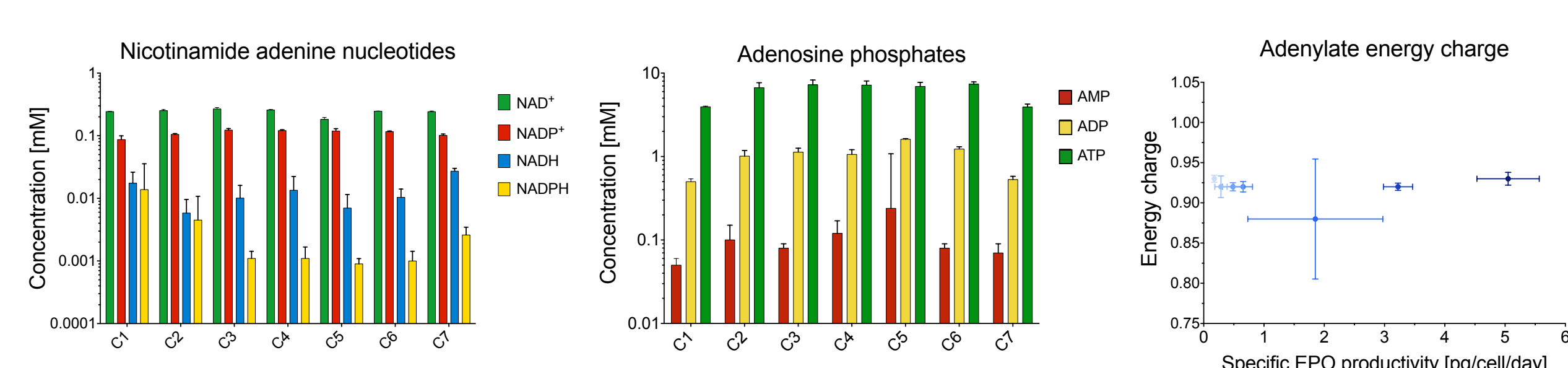
To identify differentially expressed genes functionally related to secretory protein processing across the EPO producers we performed a global gene expression analysis comparing the highest and lowest EPO producers (C7 and C1) during *phase II* (data not shown). The differential gene expression analysis identified no enrichment in the gene expression landscape of genes related to protein translocation, protein folding, protein glycosylation or vesicular transport, indicating that neither of these processes were limiting protein productivity.

Conclusion

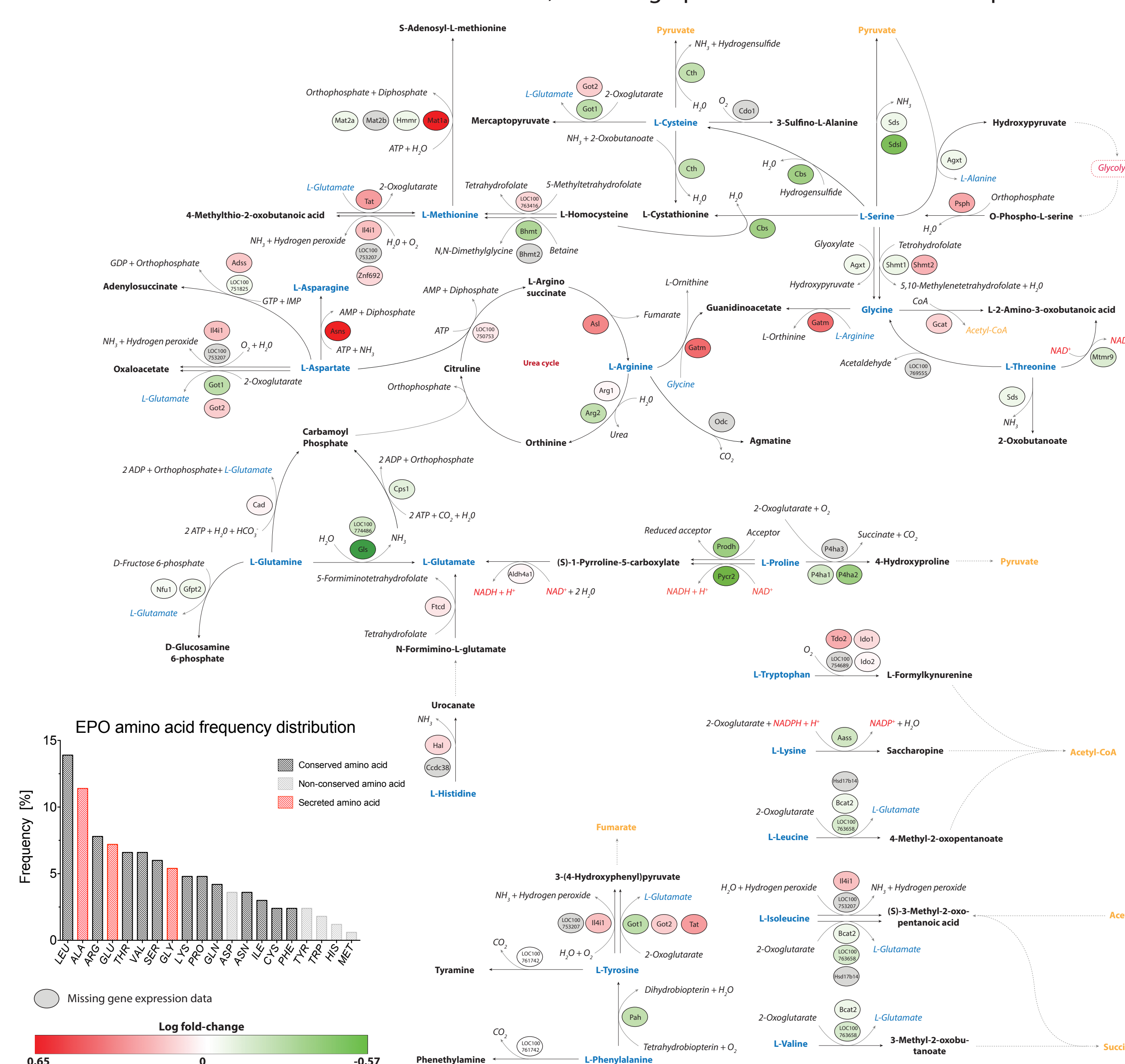
We provide evidence that EPO production up to 5 pg/cell/day is not limited by metabolism (i.e. glycolysis and associated energy metabolites) or bottlenecks in gene dosage, transcription and post-translational processing of EPO. Furthermore, we demonstrate that the amino acid catabolism can adapt to different amino acid demand, imposed by heterologous protein production, within 9 generations during prolonged chemostat cultivation.

Metabolic impact of EPO production

To discover bottlenecks in glucose metabolism, we quantified intracellular metabolites of glycolysis (data not shown) and associated energy metabolites across the EPO producing clones in mid-exponential phase in batch culture. The differences in concentration showed no correlation to specific EPO productivity, suggesting EPO production is not limited by energy supply.



To investigate the impact of EPO production on metabolism, we reconstructed glycolysis, TCA and amino acid catabolism in CHO cells (see link below) and integrated differential gene expression data from *phase II* of chemostat culture. The data integration revealed decreased transcription level of genes responsible for degradation of the amino acids most frequently found in EPO. Thus, indicating possible regulatory adaptation of gene expression towards decreased amino acid catabolism, specific for the most abundant amino acids in EPO, in the high producer relative to the low producer.



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Acknowledgements

The experimental work was carried out at Novo Nordisk in the department of Cell Culture Technology and Mammalian Cell Technology. We thank Carsten Leisted, Jens Jacob Hansen and Anja Kalløse Pedersen for experimental support.

The full metabolic network reconstruction is available here

